CRISPRi Screening in Cancer Spheroids - BME 402

Progress Report 9

Reporting Period: March 21, 2025 - April 3, 2025

Client:	Carley Schwartz	cischwartz@wisc.edu
	Dr. Gaelen Hess	ghess3@wisc.edu
Advisor:	Paul Campagnola	pcampagnola@wisc.edu
Team:	Althys Cao (Leader)	nvcao@wisc.edu
	Ana Martinez (Communicator)	almartinez4@wisc.edu
	Emily Rhine (BSAC)	erhine@wisc.edu
	Julia Salita (BWIG)	jsalita@wisc.edu
	Jayson O'Halloran (BPAG)	ohalloran2@wisc.edu

Problem statement: Although previous CRISPR screening in 2D monolayers has provided useful knowledge on cancer drivers and therapeutic susceptibilities, it lacks an element of biological relevance to an *in vivo* environment. Therefore, our team was tasked with developing a cell culture method that is compatible with a 3D environment and CRISPR screening in order to identify sources of DNA mutations in the tumor environment. Toward this end, the team must select a viable cell line for the screen, create and optimize a spheroid formation protocol, and develop a protocol to stain for γH2AX: a histone variant that is a sensitive marker for DNA damage.

Brief status update:

- 2D cell passaging
- Seed spheroids for RT-qPCR in 6 well plate
    - Ana
    - Althys
    - Julia
- Seed spheroids for γH2AX stain in 6 well plate
    - Emily
    - Jayson
- Scale up spheroids to 2 million per sample (well)

Difficulties / advice requests: The team was unable to complete our previous trial of spheroid dissociation with a 24-well plate because the Cytoflex machine malfunctioned.

Current design: Cells seeded in 24 well plate at 75k cells/cm^2 with 0.75% methylcellulose in full DMEM (10% FBS, 1% p/s).

Identity	Events	Cells/mL	Cells/ well	Starting amount of cells	Confluency (Cell#final/Cell #initial)
Full	253	28111.1111111111	67466.6666666666	142500	0.4734502924
Full-1 -tube 8	671	74555.5555555555	178933.3333333333	142500	1.2556725146
Full-2 -tube 9	1189	132111.1111111111	317066.6666666666	142500	2.2250292398
Full-3 -tube 10	826	91777.7777777778	220266.6666666667	142500	1.5457309942
Full-4 -tube 11	822	91333.3333333333	219200	142500	1.538245614
Full-5 -tube 12	854	94888.8888888889	227733.3333333333	142500	1.598128655
Full-6 -tube 13	899	99888.8888888889	239733.3333333333	142500	1.6823391813
Serum free	253	28111.1111111111	67466.6666666666	142500	0.4734502924
Serum free 1	127	14111.1111111111	33866.6666666666	142500	0.2376608187
Serum free 2	163	18111.1111111111	43466.6666666666	142500	0.3050292398
Serum free 3-tube 5	204	22666.6666666667	54400.0000000001	142500	0.381754386
Serum free 4-tube 6	280	31111.1111111111	74666.6666666666	142500	0.5239766082
Serum free 5-tube 7	315	35000	84000	142500	0.5894736842

Figure 1. Day 5 Spheroid Dissociation_Confluency Tracker_3/17/25

Materials and expenses:

D-MEM (1x) Delbecco's Modified Eagle Medium:

1. Brand: gibco
2. Volume: 500 mL
3. Content added (by us): 10% FBS (fetal bovine serum), P/S

2. Trypsin 0.05% (1x):
    1. Brand: cytiva
    2. Volume: 125 mL
3. Fetal Bovine Serum, Value FBS:
    1. Brand: gibco
    2. Volume: 500 mL
4. PBS pH 7.4 (1x):
    1. Brand: gibco
    2. Volume: 500 mL
5. A549 Cell Line

6. Poly-HEMA and Methylcellulose Sigma Aldrich Total: $289.40

Major team goals for the next week:

1. Complete all 3 qPCR steps
2. Complete first γH2AX stain
3. Meet with client and advisor to plan out the next two weeks of experiments
4. Incorporate preliminary report feedback when drafting the final report

Next week's individual goals:

- Althys Cao
    - Help team with RNA extraction, cDNA synthesis, and qPCR steps
    - Continue passaging 2D A549 WT flasks, assist with spheroid formation if needed
    - Prepare for Engineering Expo
- Ana Martinez
    - Help team with RNA extraction, cDNA synthesis, and qPCR steps
    - Continue passaging 2D A549 WT flasks
    - Continue preparing for outreach event at Engineering Expo
    - Work on my section of final report
- Emily Rhine
    - Complete first γH2AX stain. Continue passaging both 2D A549 WT flasks. Complete my section of the final report draft. Continue preparing for engineering expo outreach event and draft outreach report. Aid with qPCR steps.
- Julia Salita
    - Help team with RNA extraction, cDNA synthesis, and qPCR steps
    - Continue passaging 2D A549 WT flasks
    - Prepare our team's outreach event at Engineering Expo
    - Work on section of final report
    - Update website and lab archives
- Jayson O'Halloran
    - First yH2AX stain
    - Prep Engineering Expo
    - Start final report section
    - Passage 2D cells

Table 1. Project Timeline.

Week #	**Task**
1	**Choose project** **Assign roles**
2	**Finish first progress report** **BSAC meeting**

	First client meeting
3	**PDS, Brainstorm, Research**
4	**Brainstorm, Literature Search, Design matrix criteria and design ideas (at least three) due**
5	**Preliminary Oral Presentation**
6	**Preliminary Report, Electronic Notebook, Peer/Self Evaluation, Decide on final design**
7	**Final Design**
8	**Order materials, consider submitting invention disclosure**
9	**Fabrication, show and tell**
10	**Fabrication**
11	**Fabrication**
12	**Design Testing and Modification, Poster Draft Review**
13	**Design Testing and Modification, Final Report**
14	**Poster Presentation, Final Report, Final Electronic Notebook, Team Evaluation, Peer/Self Evaluation**

Previous week's goals and accomplishments:

- Team
  - Prepare spheroids for qPCR
  - Meet with client and advisor to plan out the next month of experiments
- Althys Cao
  - Prepare for qPCR: seed more spheroids and establish timeline
  - Start on planning gammaH2AX staining
- Ana Martinez
  - Goal: help team optimize spheroid dissociation protocol and prepare for qPCR after spring break
  - Provided advice to BME 301 students at show and tell
  - Continued passaging 2D WT A549 flasks
  - Finalized and began obtaining supplies for our BME outreach project
  - Helped draft team executive summary
- Emily Rhine
  - Optimize spheroid dissociation protocol based on previous experiments and client suggestions: 96 well and 24 well. Provide advice at show and tell to BME 301

students. Continue passaging both 2D WT A549 flasks. Attend and take notes at client meetings and team meetings to plan experiments for the next few weeks. Prepare outreach projects and final report documents.

- Julia Salita
    - Made polyHEMA stock solution.
    - Unfortunately I was sick for part of the week in the beginning and did not do as much as I wanted to and will move updating the website and lab archives to next week.
- Jayson O'Halloran
    - Spheroid dissociation
    - Continued passaging A549 2D culture
    - Updated Lab Archives

Table 2. Activities

Name	**Date**	**Activity**	**Time (h)**	**Week Total (h)**	**Sem. Total (h)**
Althys Cao	3/21 3/21 4/1 4/2 4/2 4/3	Show and tell Client meeting Team meeting 3D spheroid seeding Look at qPCR protocol Executive summary	1.5 1 1 0.5 2 0.75	6.75	60.5
Ana Martinez	3/21 3/21 4/1 4/1 4/1 4/2 4/2	Show and tell Client meeting Make polyHEMA plates Team meeting Outreach project prep Passaging 2D flasks Executive summary	1.5 1 0.25 1 1.5 1.25 0.75	6.5	52.25
Emily Rhine	3/21 3/21 3/21 3/22 3/31 4/1 4/1 4/1 4/1 3/21-4/3	Spheroid Dissociation Show and tell Client meeting Create Carley passaging plan Passage 2D WT A549 Make PolyHEMA plates Team meeting Outreach project Final report Update LabArchives	2.5 1 1 0.25 0.5 0.25 1 1 1 1	9.5	63
Julia Salita	3/21 3/21	Show and tell Client meeting	2 1	4	49

	4/1	PolyHEMA stock solution	1		
Jayson O'Halloran	3/21 3/21 3/31 4/1 4/1 4/2	Spheroid dissociation, passaging, client meeting Show and tell yH2AX 3D protocol research Team meeting Executive summary Lab archives	4 1 1 1 1 1	9	52